

CLAIMS

What is claimed is:

1. A method for determining vascular endothelial growth factor (VEGF) activity in a sample, said method comprising the steps of:
 - 5 Contacting a sample to be assayed for VEGF activity with a stable cell line comprising cells transfected with a reporter vector having an expressible reporter element and a DNA binding site disposed adjacent thereto, a chimeric transactivatable vector comprising a gene encoding a phosphorylatable protein and a DNA binding domain which specifically binds to the DNA binding site, and an expression vector encoding a gene for a VEGF receptor detecting the presence of expressed reporter element indicating VEGF activity; and
 - 10 Detecting expression of the reporter element, wherein expression of the reporter element indicates VEGF activity.
2. A method according to Claim 1, wherein the reporter vector further comprises a GAL4 binding element.
3. A method according to Claim 2, wherein the reporter vector comprises a gene encoding for a detectable product.
- 20 4. A method according to Claim 3, wherein the detectable product comprises luciferase.
5. A method according to Claim 3, wherein the gene encoding for the detectable product is operably linked to a promoter element.
6. A method according to Claim 5, wherein the promoter element comprises a TATA box.

7. A method according to Claim 1, wherein the phosphorylatable protein encoded by chimeric transactivatable vector can be phosphorylated by MAPK.
8. A method according to Claim 1, wherein the phosphorylatable protein comprises ELK-1.
9. A method according to Claim 1, wherein the gene encoding for the phosphorylatable protein is operably linked to a promoting element.
10. A method according to Claim 1, wherein VEGF receptor comprises FLK-1.
- 10 11. A method according to Claim 1, wherein the VEGF receptor encoding gene is operably linked to a promoter element.
12. A method according to Claim 11, wherein the VEGF receptor encoding gene is FLK-1.
13. A method according to Claim 1, wherein the stable cell line comprises HeLa cells.
- 15 14. A method according to Claim 1, wherein said contacting step further comprises binding VEGF present in the sample with expressed VEGF receptor.
- 20 15. A method according to Claim 14, wherein said including contacting step further comprises activating MAPK with the expressed VEGF receptor.
16. A method according to Claim 15, further comprising the step of expressing the trans-activator vector to produce a chimeric product comprising the phosphorylatable protein and DNA binding domain.

17. A method according to Claim 16, further comprising the step of phosphorylating the chimeric product with the activated MAPK.
18. A method according to Claim 17, further comprising the step of binding the phosphorylated chimeric product to the DNA binding site of the reporter vector, wherein expression of the expressible reporter element is activated indicating the presence of VEGF in the sample.
19. A method according to Claim 1, wherein the sample comprises biological fluids.
20. A method according to Claim 19, wherein the biological fluids comprise plasma or cell culture media.
21. A method according to Claim 1, wherein the sample comprises cells, tissue, tissue extracts, and combinations thereof.
22. A method according to Claim 1, wherein the VEGF activity is detectable in a concentration >1 mg/mL.
- 15 23. A method according to Claim 1, wherein the VEGF activity is detectable in a concentration range from approximately 1 ng/mL to approximately 200 ng/mL.
- 20 24. A method according to Claim 1, further comprising the step of incubating the sample with stable cell line for a period of time ranging from approximately 4 hours to approximately 24 hours.
- 25 25. A method according to Claim 1, further comprising the step of incubating the sample with stable cell line for a period of time ranging from approximately 10 hours to approximately 20 hours.
26. A method of determining whether a candidate compound is useful for modulating VEGF receptor activity, said method comprising the steps of:

(a) providing a cell expressing the VEGF receptor FLK-1;
(b) contacting the cell with a candidate compound;
(c) measuring VEGF receptor activity, wherein the altered VEGF receptor activity relative to a cell not contacted with the candidate compound, indicates that the candidate compound modulates VEGF receptor activity.

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27. A method according to Claim 26, wherein the cell further comprises a reporter vector having an expressible reporter element and a DNA binding site disposed adjacent thereto, and a chimeric transactivator vector comprising a gene encoding a phosphorylatable protein and a DNA binding domain which specifically binds to the DNA binding site.

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28. A method according to Claim 27, wherein said measuring step is further defined as comparing levels of the expressed reporter element from the cell contacted with the candidate compound relative to a cell not contacted with the candidate compound.

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29. A method for determining whether a candidate compound is useful for modulating VEGF activity, said method comprising the steps of:
(a) providing a cell expressing VEGF;
(b) contacting the cell with a candidate compound;
20 (c) contacting a sample to be assayed for VEGF activity with a stable cell line comprising cells transfected with a reporter vector having an expressible reporter element and a DNA binding site disposed adjacent thereto, a chimeric transactivatable vector comprising a gene encoding a phosphorylatable protein and a DNA binding domain which specifically binds to the DNA binding site, and an expression vector encoding a gene for a VEGF receptor detecting the presence of expressed reporter element indicating VEGF activity; and
25 (d) Detecting expression of the reporter element, wherein expression of the reporter element indicates VEGF activity.

wherein altered VEGF activity relative to a cell not contacted with the candidate compound indicates that the candidate compound modulates VEGF activity.

30. A method according to Claim 27, wherein the reporter vector further comprises a GAL4 binding element.
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31. A method according to Claim 30, wherein the reporter vector comprises a gene encoding for a detectable product.
32. A method according to Claim 31, wherein the detectable product comprises luciferase.
- 10 33. A method according to Claim 31, wherein the gene encoding for the detectable product is operably linked to a promoter element.
34. A method according to Claim 33, wherein the promoter element comprises a TATA box.
- 15 35. A method according to Claim 29, wherein the phosphorylatable protein encoded by chimeric trans-activatable vector can be phosphorylated by MAPK.
36. A method according to Claim 29, wherein the phosphorylatable protein comprises ELK-1.
- 20 37. A method according to Claim 29, wherein the gene encoding for the phosphorylatable protein is operably linked to a promoting element.
38. A method according to Claim 29, wherein VEGF receptor comprises FLK-1.
39. A method according to Claim 29, wherein the VEGF receptor encoding gene is operably linked to a promoter element.

40. A method according to Claim 39, wherein the VEGF receptor encoding gene is FLK-1.
41. A method according to Claim 29, wherein the stable cell line comprises HeLa cells.
- 5 42. A method according to Claim 29, wherein said contacting step further comprises binding VEGF present in the sample with expressed VEGF receptor.
43. A method according to Claim 42, wherein said including contacting step further comprises activating MAPK with the expressed VEGF receptor.
- 10 44. A method according to Claim 43, further comprising the step of expressing the transactivator vector to produce a chimeric product comprising the phosphorylatable protein and DNA binding domain.
45. A method according to Claim 44, further comprising the step of phosphorylating the chimeric product with the activated MAPK.
- 15 46. A method according to Claim 45, further comprising the step of binding the phosphorylated chimeric product to the DNA binding site of the reporter vector, wherein expression of the expressible reporter element is activated indicating the presence of VEGF in the sample.
47. A method according to Claim 29, wherein the sample comprises biological fluids.
- 20 48. A method according to Claim 47, wherein the biological fluids comprise plasma or cell culture media.
49. A method according to Claim 29, wherein the sample comprises cells, tissue, tissue extracts, and combinations thereof.